

Enzymatic Assay of α -CHYMOTRYPSINOGEN A

PRINCIPLE:

Chymotrypsinogen $\xrightarrow[\text{Chymotrypsin}]{\text{Trypsin}}$ Chymotrypsin + Peptide Fragments

BTEE + H₂O $\xrightarrow{\text{Chymotrypsin}}$ Na-Benzoyl-L-Tyrosine + Ethanol

Abbreviation used:

BTEE = Na-Benzoyl-L-Tyrosine Ethyl Ester

CONDITIONS: T = 25°C, pH = 7.8, A_{256nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 50 mM Potassium Phosphate Buffer, pH 8.0 at 25°C
(Prepare 100 ml in deionized water using Potassium Phosphate Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 8.0 at 25°C with 1 M KOH.)
- B. 1 mM Hydrochloric Acid Solution (HCl)
(Prepare 50 ml in deionized water using Hydrochloric Acid, Sigma Prod. No. H-7020.)
- C. 80 mM Tris HCl Buffer, pH 7.8 at 25°C (Tris Buffer)
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 7.8 at 25°C with 1 M HCl.)
- D. 1.18 mM N-Benzoyl-L-Tyrosine Ethyl Ester (BTEE)
(Prepare 50 ml using N-Benzoyl-L-Tyrosine Ethyl Ester, Sigma Prod. No. B-6125, by initially dissolving in 31.7 ml of Methanol, Absolute, Sigma Stock No. 17-5. Add enough deionized water to make a final volume of 50 ml.)
- E. 2 M Calcium Chloride Solution (CaCl₂)
(Prepare 5 ml in deionized water using Calcium Chloride, Dihydrate, Sigma Prod No. C-3881.)
- F. 0.1% (w/v) Trypsin Enzyme Solution (Trypsin)
(Immediately before use, prepare 10 ml in Reagent A using Trypsin, Type III, Sigma Prod. No. T-8253)

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REAGENTS: (continued)

- G. α -Chymotrypsinogen A Solution (Chymo A)
(Immediately before use, prepare a solution as described below in Step 1 and Step 2.)

PROCEDURE:

Step 1: Free α -Chymotrypsin Activity

Immediately before use, dissolve 10 mg of α -Chymotrypsinogen A in 10 ml of Reagent B. Immediately proceed with the chymotrypsin assay.

Step 2: Activated α -Chymotrypsin Activity

Immediately before use, dissolve 10 mg of α -Chymotrypsinogen A in 10 ml of Reagent A. To 9 ml of this solution, add 1 ml of Reagent F (Trypsin) and incubate at 25°C for 3 hours. Then dilute 0.5 ml of this solution to 10.5 ml with Reagent B. Proceed with the chymotrypsin assay.

Step 3: Chymotrypsin Assay

Pipette (in milliliters) the following reagents into suitable cuvettes:

	<u>Test</u>	<u>Blank</u>
Reagent C (Tris Buffer)		1.42
		1.42
Reagent D (BTEE)	1.40	1.40
Reagent E (CaCl ₂)	0.08	0.08

Mix by inversion and equilibrate to 25°C. Monitor the $A_{256\text{nm}}$ until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent B (HCl)	-----	0.10
Enzyme Solution (from Step 1 or Step 2)	0.10	-----

Immediately mix by inversion and record the increase in $A_{256\text{nm}}$ for approximately 5 minutes. Obtain the $\Delta A_{256\text{nm}}/\text{minute}$ using the maximum linear rate for both the Test and Blank.

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CALCULATIONS:

The calculations shown below can be used to determine both the free α -Chymotrypsin activity and also the α -Chymotrypsin activity after activation by trypsin.

$$\text{Units/ml enzyme} = \frac{(\Delta A_{256\text{nm}}/\text{min Test} - \Delta A_{256\text{nm}}/\text{min Blank})(3)(\text{df})}{(0.964)(0.1)}$$

3 = Volume (in milliliters) of assay

df = Dilution factor

0.964 = Millimolar extinction coefficient of N-Benzoyl-L-Tyrosine at 256 nm

0.1 = Volume (in milliliter) of enzyme used in assay

$$\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$

$$\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

UNIT DEFINITION:

One unit will hydrolyze 1.0 μ mole of BTEE per minute at pH 7.8 at 25°C.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 38 mM Tris, 0.55 mM N-benzoyl-L-tyrosine ethyl ester, 30% (v/v) methanol, 53 mM calcium chloride, 0.03 mM hydrochloric acid, 0.48 μ g trypsin, and 4.3 μ g of α -chymotrypsinogen A.

REFERENCES:

Rick, W. (1974) in *Methods of Enzymatic Analysis*, 2nd ed., Volume II, pp 1009-1012, Academic Press Inc., New York, NY

NOTES:

1. This assay is based on the cited reference.
2. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

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This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.